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CUTANEOUS LIPOGENESIS. MAJOR PATHWAYS OF CARBON FLOW AND POSSIBLE INTERRELATIONSHIPS BETWEEN THE EPIDERMIS AND SEBACEOUS GLANDS*

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Since the major pathways of lipid synthesis have been elucidated in various organisms (Shrago et al., 1971), it is unlikely that they should differ essentially in skin. Nevertheless, certain features of skin lipids seem peculiar and need to be explained. Skin surface lipids contain appreciable amounts of three types of compounds which are absent from or present only in trace amounts in the lipids of other tissues: (1) branched-chain and odd-numbered fatty acids, both free and as esters (James and Wheatley, 1956; Wheatley and James, 1957); (2) diester waxes, of which there are two types (Nicolaides et al., 1970); esters formed from an α-hydroxy fatty acid by the combination of the carboxyl group with a wax alcohol and the hydroxyl group to another fatty acid, and esters of fatty acids with a long-chain alkane-diol; (3) intermediates in the pathway of cholesterol synthesis which range from squalene to lathosterol with the sterols in both free and esterified forms (Kandutsch, 1964). How and why are these compounds formed?

Another feature of skin must be considered. In most animal tissues, lipogenesis converts excessive dietary carbohydrate or protein into storage lipid (Masoro, 1968), and this process must obviously be under the control of homeostatic regulatory mechanisms related to dietary intake and composition. The lipids of the skin, both sebaceous and epidermal, however, are formed for a specific biologic function, to maintain the integrity of the integument. This poses a problem. Is lipogenesis in skin regulated by the same mechanisms that operate in other tissues? If it is, then these very mechanisms jeopardize its basic function; if it is not, then how does it avoid such regulation? We must find answers to these basic questions before we can begin to understand cutaneous lipogenesis and to devise ways in which to regulate it artificially.

If we assume that lipids are formed in the skin by the same basic mechanisms used by other organisms and tissues (Masoro, 1968), specifically that fatty acid chains are formed by elongation processes involving acetyl-CoA and reductive hydrogens and that cholesterol is formed from the same basic precursors, then certain questions must be answered.

1. What is the source of the acetyl-CoA and how is it translocated to the lipogenic sites in the skin cell?
2. What is the source of the reductive hydrogens?
3. What is the source of the branched and odd-numbered portions of the skin lipid aliphatic chains?
4. Can we delineate, with reasonable accuracy, the major pathways of carbon flow during cutaneous lipogenesis?

An attempt has been made to answer these questions. For these studies slices from the ear skin of the guinea pig have been used because they have several advantages: ear skin is virtually free from dermal adipocytes, and furthermore the adipocyte of the guinea pig, unlike that of the rat but like that of man, is unable to synthesize fatty acids. Admittedly it is not human skin and species differences must be considered; nevertheless it has enabled us to establish certain basic facts about skin and thereby has greatly simplified any subsequent study of human skin. Cutaneous lipogenesis occurs at two major sites in the skin, the epidermis and the sebaceous gland. In this study we have not tried to separate these but have studied skin in toto.

METHODOLOGY

Most of these experiments were performed on 10-mg slices of guinea pig ear, obtained by the slice technique (Wheatley et al., 1970, 1971). In experiments where incorporation rates of carbon dioxide were measured or where the tissue was preincubated before the addition of the substrate, reaction flasks (Kontes Glass Co., K882300) containing 1 ml of medium and 20 mg of tissue were used. Except for time-course studies, all tissues were incubated for 4 hr at 37° C and, except where indicated, all media contained 11.1 mM glucose.

At the end of incubation, the skin slices were transferred to test tubes containing alcoholic potassium hydroxide, saponified overnight, and acidified with hydrochloric acid; the lipids were then extracted with hexane. After the hexane extract had been washed free from radioactive precursor, an aliquot was set aside for radioactivity assay and the remainder was separated into fatty acids and nonsaponifiables by thin-layer chromatography. The separated fractions were then assayed for radioactivity.

Most of the samples were counted in a Nuclear-Chicago UNILUX liquid scintillation counter. Quench-efficiency corrections for ^{14}C were made by the channels ratio method (Bush, 1963) and for ^{3}H by means of an internal standard. In later experiments, samples were counted in an Intertechnique SL40 liquid scintillation counter with quench-efficiency corrections made by external standardization.

In certain experiments, lactate was assayed by the method of Barker and Summerson (1941).

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PRECURSOR UTILIZATION

A broad-scope survey was made of precursors that could be used by the skin for lipid synthesis. Representative examples of the following classes of compounds were studied: short-chain fatty acids, glucose and glycolysis products, Krebs cycle acids, and amino acids. Incorporation rates were expressed as nonomoles (nmoles) of precursor incorporated into fatty acids and nonsaponifiables per gram of tissue per hour of incubation and were uncorrected for loss of radioactive carbon atoms during metabolism. The results are summarized in Table I.

The short-chain fatty acids—acetic, propionic, and butyric—were actively incorporated into lipids, both fatty acids and nonsaponifiables, as were glucose, pyruvate, and lactate. Of the amino acids, alanine and the branched-chain amino acids leucine and isoleucine were actively incorporated. Valine showed moderate incorporation. The rates for aspartate and glutamate were lower than anticipated, and the incorporation of other amino acids was negligible.

The results for amino acids differ from data previously obtained by the dog skin perfusion technique (Wheatley et al., 1967). This suggests that skin in vivo has the ability to select the amino acids it requires to synthesize specific lipids. This has been amply confirmed by the dietary studies of Grigor et al. (1970).

TABLE I

Incorporation of precursors into lipids by guinea pig ear skin slices

Precursor	Rate of incorporation into:	
	Fatty acids (nmoles/gm/hr)	Nonsaponifiables (nmoles/gm/hr)
Acetate-1,2-^{14}C	63.9 ± 5.2*	20.3 ± 3.4
Propionate-1-^{14}C	49.5 ± 5.7	6.5 ± 0.9
Butyrate-1-^{14}C	147.2 ± 17.4	29.2 ± 1.7
D-Glucose-U-^{14}C	53.5 ± 8.9	16.6 ± 3.0
Pyruvate-2-^{14}C	104.0 ± 6.4	30.4 ± 2.8
D,L-Lactate-2-^{14}C	55.4 ± 9.3	28.9 ± 2.7
Citrate-1,5-^{14}C	4.6 ± 0.5	1.8 ± 0.1
Succinate-2,3-^{14}C	7.0 ± 0.5	3.4 ± 0.2
L-Alanine-U-^{14}C	90.7 ± 11.8	18.8 ± 1.9
L-Aspartate-U-^{14}C	10.2 ± 0.6	2.5 ± 0.1
L-Glutamate-U-^{14}C	6.8 ± 1.7	2.1 ± 0.3
Glycine-1,2-^{14}C	1.2 ± 0.2	ns**
L-Isoleucine-U-^{14}C	34.5 ± 1.6	6.9 ± 0.3
L-Leucine-U-^{14}C	70.4 ± 5.6	37.6 ± 5.0
L-Lysine-U-^{14}C	2.3 ± 0.1	ns
L-Methionine-(Me-^{14}C)	1.6 ± 0.3	ns
L-Phenylalanine-U-^{14}C	3.1 ± 0.2	1.8 ± 0.2
L-Valine-U-^{14}C	11.4 ± 1.7	2.9 ± 0.4

* Mean and standard error of triplicate incubations performed on ear slices from at least two different animals.

** Potential errors of the method indicate that, in these incubations, rates below 1.0 are not significant and have been designated *ns*.

The plasma membranes of skin cells are almost impermeable to citrate; not only is citrate taken up poorly by the cells (Barron et al., 1948) but citrate formed by the cells accumulates inside them (Firscheim and Bell, 1961). Hence the incorporation rates observed for citrate are low.

GENERATION AND TRANSLOCATION OF ACETYL-COA

Data from precursor utilization (Table I), mitochondrial inhibition (Wheatley et al., 1971), and other studies (Mier, 1969; Decker, 1971) are consistent with the generation of acetyl-CoA in the mitochondria by mechanisms similar to those that are active in other tissues. This acetyl-CoA could be generated from pyruvate formed by glycolysis, by catabolism of amino acids, and by β-oxidation of fatty acids. Undoubtedly some acetyl-CoA is generated by the catabolism of endogenous amino acids. However, there is an almost hundredfold increase in the mass of the sebaceous cell during lipogenesis (Wheatley et al., 1971); hence, at this site, the bulk of the precursors for lipogenesis must be provided by the circulating tissue fluids. Observations, cited above, indicate that circulating amino acids are used by skin to provide other acyl-CoA compounds in preference to acetyl-CoA; it seems unlikely, therefore, that much of the acetyl-CoA generated by skin is derived from exogenous amino acids. Although acetyl-CoA is unquestionably generated from fatty acids in skin (Cruickshank et al., 1962), this would be a wasteful process in lipogenesis and is more likely to be involved only with energy production. Furthermore, unpublished experiments performed in these laboratories (Wheatley and Hodgins, 1968) indicate that circulating fatty acids are taken up predominantly by dermal adipocytes with little uptake by the epidermal or sebaceous cells. Most of the acetyl-CoA used by skin for lipogenesis seems to be generated in the mitochondria from the pyruvate provided by glycolysis. Thus, in skin, as in other tissues, lipogenesis is intimately linked with carbohydrate metabolism.

Three major processes of chain elongation are used in fatty acid synthesis: de novo, microsomal, and mitochondrial (Donaldson et al., 1970). The de novo and microsomal processes take place extramitochondrially; hence we must establish how the acetyl-CoA required is translocated from the mitochondria to the cytosol. Several possible mechanisms have been recognized (Lowenstein, 1968): cleavage to acetate with subsequent re-formation in the cytosol, carnitine-mediated translocation, the citrate cleavage pathway (see Fig. 1), as unchanged acetyl-CoA. Mitochondria of other tissues are impermeable to acetyl-CoA. If translocation is carnitine mediated, then D,L-, or L-carnitine

TABLE II

Effects of carnitine, deoxycarnitine, acetylcysteine, and hydroxycitrate on the incorporation rates of acetate and glucose

Precursor	Effector	Concentration (mM)	Incorporation rate as a percentage of control incubation		
			Fatty acids	Nonsaponifiables	Carbon dioxide
Acetate-1,2-^{14}C	D,L-Carnitine	5.0	71.5*	69.7	80.2
	Acetylcysteine	2.0	39.5	54.3	—
D-Glucose-U-^{14}C	D,L-Carnitine	5.0	102.6	97.0	—
	L-Carnitine**	5.0	113.9	94.4	—
	Deoxycarnitine	5.0	85.5	81.9	—
	Acetylcysteine	2.0	192.2	137.0	—
	Hydroxycitrate**	5.0	17.5	16.3	—

* Means of triplicate incubations performed on two different animals.

** A generous supply of L-carnitine was made available by the Otsuka Pharmaceutical Factory, Osaka, Japan. Hydroxycitrate was supplied by Hoffman-La Roche, Nutley, New Jersey.

should stimulate lipogenesis from glucose, and deoxycarnitine should inhibit it; carnitine should also stimulate the production of carbon dioxide from acetate. If translocation is achieved by citrate cleavage, then lipogenesis from glucose should be inhibited by (—)-hydroxycitrate (Sullivan et al., 1972). If translocation is achieved as free acetate, then acetylcysteine should inhibit lipogenesis from both acetate and glucose, since this compound appears to inhibit acetyl-CoA synthetase (EC 6.2.1.1: Wheatley et al., unpublished data). The results of the study of these effector compounds are shown in Table II. Both carnitine and deoxycarnitine are without effect, acetylcysteine inhibits only acetate incorporation, and hydroxycitrate is a powerful inhibitor of lipogenesis from glucose. On the basis of these observations, we concluded that the major pathway of translocation is by citrate cleavage. Neither of the alternative mechanisms seems to be operative in skin, but they cannot be excluded completely on the basis of these data alone. Moreover, hydroxycitrate does not completely inhibit lipogenesis in skin. The residual low rate of lipogenesis could be cited as evidence for the operation of alternative mechanisms functioning at a lower rate than citrate cleavage, but this residual is more likely mitochondrial lipogenesis.

Once the citrate cleavage pathway is accepted as a major mechanism of translocation of acetyl-CoA, then certain specific questions must be answered. For an active flow of citrate from the mitochondria to be maintained, there must be a source of mitochondrial oxaloacetate. Furthermore, as a result of citrate cleavage, oxaloacetate is formed in the cytosol but its fate is not known. The mitochondria of all tissues so far studied are impermeable to oxaloacetate (Chappell, 1968), and unless evidence to the contrary is forthcoming, we must assume that the same applies to the mitochondria of the epidermal and sebaceous cells. These points will be further considered in the next section.

GENERATION OF REDUCTIVE HYDROGENS

In addition to acetyl-CoA, a source of reductive hydrogens must be provided before lipogenesis can proceed. Regardless of the mechanism involved, a total of four reductive hydrogens is required by each acetyl unit added. These reductive hydrogens are provided by $NADPH_2$† in the de novo process, by either $NADPH_2$ or $NADH_2$† in the microsomal mechanisms, and by $NADH_2$ in the mitochondrial process. Since there is ample $NADH_2$ available for the latter process, we are mainly concerned with the generation of extramitochondrial $NADPH_2$. As will be seen later, cytosolic $NADH_2$ may play a role, and direct transport of reductive hydrogens into and out of mitochondria is unlikely (Chappell, 1968; Robinson and Halperin, 1970).

Three major mechanisms for the generation of $NADPH_2$ have been recognized (Hanson et al., 1971): isocitrate dehydrogenase (ICDH-NADPH; EC 1.1.1.42), the malate cycle‡, and the hexose-monophosphate shunt (HMP-shunt). These pathways are illustrated in Figure 1.

The standard method for measuring the activity of the HMP-shunt compares the incorporation of glucose-1-^{14}C with glucose-6-^{14}C into either carbon dioxide or lipid. Abraham and Chaikoff (1959)

† These abbreviations have been used for reduced nicotinamide-adenine dinucleotide and nicotinamide-adenine dinucleotide phosphate, respectively, and indicate the additional hydrogen derived as a proton from water. During elongation, one of the reductive hydrogens added at the first step is eliminated as a hydroxyl hydrogen in a subsequent step. There is, in fact, considerable randomization of the hydrogens during the entire process. Though theoretically 10 of the 31 hydrogens of palmitoyl-CoA should come from acetyl-CoA, 14 from $NADPH_2$, and 7 from water (Foster and Bloom, 1963), experimentally 23 are derived from water (Jungas, 1968).

‡ Flatt (1970) defines the malate cycle as the sequence of reactions from oxaloacetate to malate to pyruvate to oxalocetate, catalyzed by malate dehydrogenase (EC 1.1.1.37), malic enzyme (EC 1.1.1.40), and pyruvate carboxylase (EC 6.4.1.1).

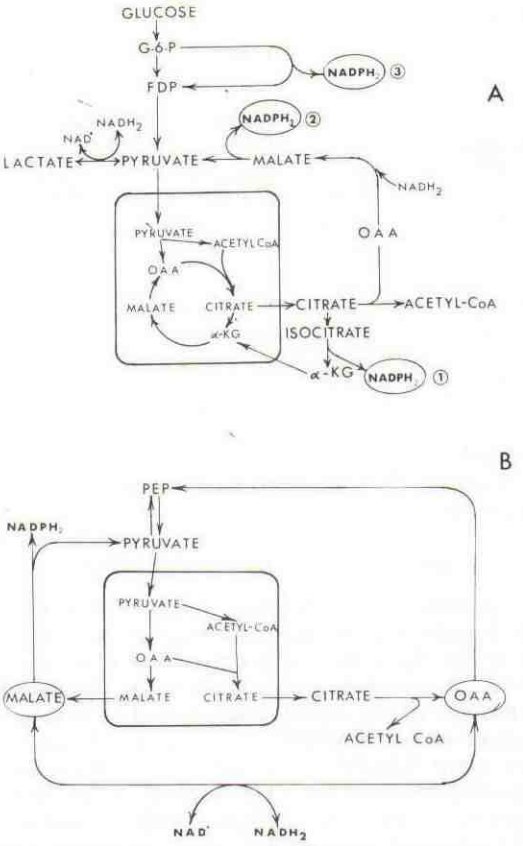

FIG. 1: (A) Generation of $NADPH_2$ by (1) isocitrate dehydrogenase, (2) malate cycle, and (3) hexose monophosphate shunt. (B) Alternative pathway for recycling oxaloacetate which does not require cytosolic $NADH_2$. The mitochondrial compartment is represented by the box. *Abbreviations:* G-6-P, glucose-6-phosphate; FDP, fructose diphosphate; OAA, oxaloacetate; α-KG, α-ketoglutarate.

criticized the measurement of incorporation into carbon dioxide because of the errors introduced by recycling and proposed that measurement of incorporation into lipid gives more reliable results. In the present series of studies (Wheatley et al., 1971), identical rates of incorporation of glucose-1-^{14}C and glucose-6-^{14}C were observed that were difficult to explain since the HMP-shunt is active in the skin of the guinea pig ear (Yardley and Godfrey, 1963). Later we realized that this discrepancy was the result of the enormously high rate of lactate production in skin, which accounts for some 80 percent of the total glucose flux (Freinkel, 1960). The relative flow of the HMP-shunt is too low to be measured by this ratio method (Landau and Katz, 1965). Recourse to the use of carbon dioxide incorporation rates was contraindicated by the observation of significant carbon dioxide fixation processes in skin (vide infra). Therefore, we attempted to establish the relative contributions of the three pathways by indirect methods. In the absence of glucose, acetate can generate $NADPH_2$ only by the ICDH–NADPH pathway since it cannot generate the oxaloacetate to operate either the citrate cleavage pathway or the malate cycle. It could do this only if it could enter the mitochondria. Evidence for this entry could be obtained by measuring the incorporation of acetate into carbon dioxide; hence this was a first requirement. In the absence of glucose, pyruvate can generate $NADPH_2$ by both the ICDH–NADPH and the malate cycle pathways. With both acetate and pyruvate, the HMP-shunt is inoperative. The malate cycle operates by recycling the cytosolic oxaloacetate resulting from citrate cleavage, but for this a source of cytosolic $NADH_2$ is required (Fig. 1A). It is difficult to see how pyruvate can supply this, but it can easily be supplied if lactate is used as precursor. The incorporation rates of these three precursors in the absence of glucose were compared in various ways to corroborate the data. Either the incubation was performed with media containing no added glucose, or the tissue was preincubated for 1 hr to exhaust all endogenous glucose and glycogen, and the substrate (without glucose) was added. Time–course studies were undertaken to demonstrate changes in incorporation rates when the endogenous glucose was exhausted. The effect of pyruvate on lipid incorporation from tritiated water was also studied, since this provides an independent method of measuring the rates of lipogenesis (Jungas, 1968). The results of these studies are presented in Table III and Figure 2.

Active incorporation of acetate into carbon dioxide was observed (Table III); since this was unchanged in the presence of glucose, skin mitochondria are readily permeable to acetate, and carnitine (Table II) is not involved in its transport. Thus we can assume that the ICDH–NADPH pathway is active. Time–course studies with acetate and pyruvate (Fig. 2) show an initial high rate of incorporation of both precursors before endogenous glucose and glycogen are exhausted; the rate then falls to a steady level for the remainder of the incubation period. As would be expected, pyruvate is better able to conserve endogenous glucose. Hence with acetate, an initial rate of incorporation into fatty acids of 24.7 nmoles/gm/hr is maintained for the first half hour only, when the rate falls to 10.1 nmoles/gm/hr and remains steady for the remaining 3 hr of incubation. With pyruvate, the initial lag in uptake seen in other time–course experiments (Wheatley et al., 1971) is more obvious. The incorporation rate starts at 26.2 nmoles/gm/hr for the first half hour, rises to 100.8 nmoles/gm/hr for the next half hour, then settles to a steady 56.2 nmoles/gm/hr after 2 hr. It is quite clear from these curves that incorporation of both acetate and pyruvate can proceed after all exogenous glucose is exhausted, and the data presented in Table III substantiate this. The rate is low for acetate, but appreciable for pyruvate. Thus, in skin, both the ICDH–NADPH and malate cycle pathways are active.

For triglycerides to be formed (these are actively

TABLE III

Studies of the generation of $NADPH_2$

Precursor and incubation conditions	Incorporation rate into: Fatty acids (nmoles/gm/hr)	Nonsaponifiables (nmoles/gm/hr)	Carbon-dioxide (nmoles/gm/hr)
Standard incubation, no glucose			
Acetate-1,2-^{14}C (2 mM)	10.0 ± 2.0	7.3 ± 1.4	—
Pyruvate-2-^{14}C (5 mM)	20.4 ± 2.1	15.4 ± 1.7	—
D,L-Lactate-2-^{14}C (2 mM)	31.8 ± 5.0	16.1 ± 2.1	—
L-Lactate-2-^{14}C (5 mM)	48.4 ± 14.3	10.4 ± 0.9	—
D-Glucose-U-^{14}C (5 mM)	47.5 ± 7.1	15.6 ± 2.1	—
Pre-incubation, no glucose			
Acetate-1,2-^{14}C	19.3 ± 2.4	15.2 ± 0.7	—
Pyruvate-2-^{14}C	103.4 ± 15.0	37.7 ± 1.5	—
D,L-Lactate-2-^{14}C	71.7 ± 3.1	19.7 ± 0.7	—
Carbon dioxide output from acetate-1,2-^{14}C			
* Without glucose	6.4	7.5	331.5
* With glucose	29.0	21.7	325.1
Incorporation of tritiated water			
Control	60.6 ± 7.3	61.9 ± 7.3	—
With pyruvate (10 mM)	103.7 ± 5.4	93.6 ± 4.5	—

Except where indicated by an asterisk (*) the above results represent mean and standard error of triplicated incubation of ear slices from the same animal. Other incubations were performed in duplicate and mean only is given.

formed by skin slices. Wheatley et al., 1971), a source of L-α-glycerophosphate (α-GP) is required (Howard and Lowenstein, 1965). In the absence of glucose, pyruvate can generate this by the reversal of glycolysis to dihydroxyacetone phosphate (DHAP) and the formation of α-GP from the DHAP so generated. This process, which was called glycerogenesis by Hanson and his colleagues (Patel et al., 1971), requires cytosolic $NADH_2$. The generation of α-GP may be rate-limiting in lipogenesis (Ziboh and Hsia, 1969) and is inoperative when acetate, without glucose, is the precursor. Furthermore, no assessment of the extent of cutaneous mitochondrial lipogenesis is yet available; this would be independent of both cytosolic acetyl-CoA and reductive hydrogens. Hence, we cannot

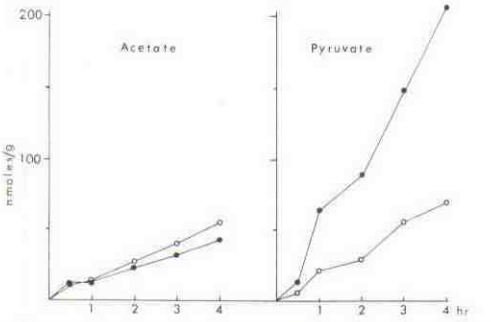

FIG. 2: Time-course studies of the incorporation of 2 mM acetate and 2 mM pyruvate into fatty acids (●) and nonsaponifiables (○) by guinea pig ear skin slices in media containing no glucose.

say that the rate from acetate is due to the ICDH-NADPH pathway alone and that the rate from pyruvate is equal to the sum of the ICDH-NADPH and malate cycle pathways. We are, therefore, unable to accurately compute the relative contribution of the former pathway.

As pointed out previously, like the generation of α-GP, recycling of the oxaloacetate liberated by citrate cleavage through the malate cycle requires a source of cytosolic $NADH_2$. Lactate can supply this $NADH_2$ and, as can be seen from Table III, lipogenesis from lactate is more active than from pyruvate and almost as active as from glucose. (Since D-lactate is inactive and the rate of racemization probably low, the rate of incorporation of D,L-lactate is about half that for L-lactate; moreover, one mole of lactate generates only one mole of acetyl-CoA but one mole of glucose generates two; thus for purposes of comparison the rates for D,L-lactate and D-glucose should be doubled.) This suggests that in skin, in contrast to other tissues (Flatt and Ball, 1864), the HMP-shunt is relatively less important as a mechanism for generating $NADPH_2$ for lipogenesis. There are still inconsistencies in our data and it is difficult to assess the relative contributions of the three pathways at this time. A conservative estimate would be: ICDH-NADPH pathway, 10 percent; malate cycle, 40 percent; HMP-shunt, 50 percent.

The role of cytosolic $NADH_2$ in the regulation of lipogenesis in skin requires further elucidation. If, when pyruvate is the precursor, recycling of cytosolic oxaloacetate through the malate cycle is inoperative, then the cytosolic oxaloacetate probably recycles through phosphoenol pyruvate (PEP) (Fig. 1B). This pathway has been proposed by Kneer and Ball (1968) but is regarded by Hanson et al. (1971) to be relatively inactive in adipose tissue since PEP-carboxykinase (EC 4.1.1.32) does not increase in activity in response to the adaptive dietary changes that induce lipogenesis. Since lipogenesis in skin does not appear to respond to these adaptive changes, this objection may not apply. Malate dehydrogenase is reversible and the cytosolic malate/oxaloacetate equilibrium could be a rate-limiting process and supply either NAD or

$NADH_2$ as required. The role of this equilibrium has been discussed by Garland (1968), and how it functions in skin should be considered.

SPECIAL PROBLEMS

Acetate and Propionate

Both acetate and propionate are actively utilized by skin for lipogenesis, but in order to elucidate how they do so, we studied the relative rates of incorporation of specific carbon atoms. Acetate can be metabolized in various ways. It can form acetyl-CoA directly by the action of the enzyme acetyl-CoA synthetase, in which case both carbons will be incorporated at equal rates. It can enter the mitochondria, form citrate, and re-form acetyl-CoA after translocation and citrate cleavage. Because of the stereospecificity of the citrate cleavage enzyme (EC 4.1.3.8), the acetyl-CoA and oxaloacetate liberated on cleavage are identical with those originally condensed. Hence, by this pathway the two carbon atoms are also incorporated at equal rates. Finally the acetate can enter the Krebs cycle, where C-2§ is lost as carbon dioxide at the first pass, leaving C-1 as carboxyl on oxaloacetate. If the latter reaches the cytosol and is recycled, by any mechanism whatever, C-1 is lost as carbon dioxide when the oxaloacetate is converted into acetyl-CoA. In the latter case, neither of the carbon atoms is used in lipogenesis. Since, in the last mechanism, C-2 is lost as mitochondrial carbon dioxide and C-1 as cytosolic carbon dioxide, any difference in the rates of incorporation of acetate-1-^{14}C and acetate-2-^{14}C would reflect different carbon dioxide fixation processes. However, in other tissues, carbon dioxide fixation plays no role in lipogenesis (Feller, 1954).

Propionate can be incorporated in toto as a C_3-unit to form odd-chain acids (James et al., 1956) or can lose either C-1 or C-3 to form a C_2-unit which is incorporated; thus the incorporation rate or propionate-1-^{14}C must be less than that of propionate-2-^{14}C (Katz and Kornblatt, 1962).

The results of our studies on specifically labelled acetates and propionates (Table IV) are surprising and difficult to explain. Not only is acetate-2-^{14}C incorporated at a significantly higher rate than acetate-1-^{14}C, but propionate-1-^{14}C is incorporated at more than double the rate for propionate-2-^{14}C. Unquestionably, propionate is not only used by skin to form odd-chain acids (Wheatley et al., 1971) but also metabolized in other ways, but no known pathway of propionate metabolism explains the discrepancy. Recent work by Hodgins and Wheatley (unpublished) indicates that the C-1 of propionate forms a C_2-unit with a carbon atom from another molecule and that this C_2-unit is incorporated into lipid, but experimental proof of the pathway involved has yet to be obtained. The acetate data suggest fixation of carbon dioxide formed in the mitochondria, but here again further elucidation is needed.

Carbon Dioxide Fixation

The results just described suggested that there may be significant carbon dioxide fixation into lipid by the skin; hence further studies were performed to determine whether this process was general or restricted to short-chain fatty acids. The incorporation of various carboxyl-labelled precursors was studied and the results are shown in Table V. The rate for pyruvate-1-^{14}C is close to the limit of significance of the method and represents insignificant incorporation. The incorporation of the carboxyl carbons of succinate and isoleucine, and also of asparate and glutamate (see Table IX), is negligible. On the other hand, the carboxyl-carbons

TABLE IV

Incorporation of acetate and propionate

Precursor	Incorporation rate into:	
	Fatty acids (nmoles/gm/hr)	Nonsaponifiables (nmoles/gm/hr)
Acetate-1,2-^{14}C	63.9 ± 5.2*	20.3 ± 3.4
Acetate-1-^{14}C	63.8 ± 4.8**	28.7 ± 5.1
Acetate-2-^{14}C	81.6 ± 8.5**	44.1 ± 6.7
Propionate-1-^{14}C	49.5 ± 5.7	6.5 ± 0.9
Propionate-2^{14}C	21.1 ± 4.6	3.5 ± 0.5

* As in Table I.

** Analysis of the data from individual experiments shows that this difference is highly significant.

TABLE V

Carbon dioxide fixation by the skin from certain precursors

Precursor	Incorporation rate into:	
	Fatty acids (nmoles/gm/hr)	Nonsaponifiables (nmoles/gm/hr)
Pyruvate-1-^{14}C	1.3 ± 0.3*	1.6 ± 0.1
Pyruvate-2-^{14}C	104.0 ± 6.4	30.4 ± 2.8
Succinate-1,4-^{14}C	ns*	ns
Succinate-2,3-^{14}C	7.0 ± 0.7	3.4 ± 0.2
Isoleucine-1-^{14}C	ns	ns
Isoleucine-U-^{14}C	39.5 ± 1.6	6.9 ± 0.3
Leucine-1-^{14}C	4.0 ± 0.3	2.9 ± 0.2
Leucine-U-^{14}C	44.2 ± 4.4	23.3 ± 1.6
Valine-1-^{14}C	6.5 ± 1.1	1.8 ± 0.2
Valine-U-^{14}C	10.0 ± 1.6	1.7 ± 0.3
Bicarbonate-^{14}C	7.0 ± 0.6	7.0 ± 0.6

* As in Table I.

§ To avoid confusion, the following abbreviations have been used. The number of the carbon atom in a chain is indicated by a suffix numeral, whereas a chain of carbon atoms of given length is indicated by a subscript numeral. Thus C-3 indicates the third carbon atom in the chain, while C_3 means a chain three carbon atoms long.

of leucine and valine are actively incorporated, and even bicarbonate alone shows significant incorporation. There is thus significant carbon dioxide fixation into lipids by the skin. Although the mechanism involved remains to be elucidated, it does not seem to be a single process. The rate for propionate-1-^{14}C is too high to be accounted for by carbon dioxide fixation alone, and the behavior of the amino acids does not conform to their known metabolism. Thus, the transaminases acting on all three branched amino acids are the same (Taylor and Jenkins, 1966), and the decarboxylases of leucine and isoleucine are identical (Bowden and Connelly, 1968) and differ from that for valine, yet the results indicate that isoleucine and not valine is handled in a different manner. Furthermore, since the catabolism of propionate and the amino acids is essentially mitochondrial, a rather specialized process of carbon dioxide fixation may occur in skin mitochondria.

Branched-Chain Synthesis

There is ample evidence that the *iso*- and *anteiso*-branched-chain fatty acids of skin are formed from the amino acids leucine, isoleucine, and valine (Wheatley et al., 1961, 1971; Grigor et al., 1970) and there is some evidence that, in other tissues, the amino acid forms the terminal end of the fatty acid (Horning et al., 1961). Unequivocal evidence for such a mechanism in skin is lacking; moreover, branching could result from the introduction of methylmalonyl-CoA derived from propionyl-CoA, one of the products of isoleucine catabolism (Wheatley et al., 1971). In an attempt to resolve this problem, Hodgins, ¶ working in the author's laboratory, studied the incorporation of ^{14}C-valine by skin slices of mouse ear. He showed appreciable incorporation into the *iso*-acids and reported that when the latter were subjected to oxidative cleavage, only 25 per cent of the radioactivity was found in the straight-chain aldehyde liberated. He was, however, unable to isolate the acetone liberated at the same time and to confirm that most of the activity was in the terminal branched portion. Neither was he able to establish unequivocally that he was dealing exclusively with *iso*-acids, but relied on gas chromatography retention times for identification. Nevertheless, the evidence strongly suggests that the amino acids are catabolized (Fig. 3) to form branched acyl-CoA compounds which are subsequently elongated by the addition of acetyl-CoA or malonyl-CoA units.

If this is, indeed, the metabolic pathway, it raises an interesting point. The amino acid catabolism is essentially mitochondrial; hence isobutyryl-CoA, isovaleryl-CoA, α-methyl-β-butyryl-CoA, and methylmalonyl-CoA are formed in the mitochondria. But how are these acyl-CoA units elongated? If the mechanism is mitochondrial, there is no problem, and the estimate of 15 percent mitochondrial fatty acid synthesis, suggested by the hydroxycitrate inhibition studies (see Table II), would be sufficient to account for the proportion of branched-chain compounds found in guinea pig skin. On the other hand, if either the de novo or elongation mechanisms are involved, then mechanisms for translocating these acyl-CoA compounds to the cytosol must be postulated. Translocation in the form of the free acids, with cytosolic re-formation, is the only feasible mechanism to suggest at this time.

The Role of Lactate

The major metabolite of glucose metabolism by the skin is lactate (Cruickshank and Trotter, 1956; Freinkel, 1960). Data obtained in my laboratory on the distribution of incorporated activity from glucose (Table VI) agree in general with the results of other investigators. Perhaps the most striking is the high lactate concentration reached in the media and tissue by the end of the incubation period. Most of this lactate is formed by the epidermis (Halprin and Ohkawara, 1966) and its fate in vivo is the subject of considerable speculation. Since lactate is actively utilized by skin for lipogenesis, it is inevitable, in view of the high lactate levels reached in the tissues in vitro, that some of the lactate generated by the epidermis during slice incubation should be used by the sebaceous glands for lipogenesis. It now remains to establish how this relates to the situation in vivo. To what extent is epidermal lactate utilized by the sebaceous glands in preference to other precursors? The answer cannot be arrived at by means of the techniques described here; other approaches will have to be used.

As a precursor for lipogenesis, lactate has advantages because it provides cytosolic $NADH_2$ for glycerogenesis and for the malate cycle. Other investigators (Halperin and Robinson, 1970; Katz and Wals, 1970) have reasoned that the rate of generation of $NADH_2$ from lactate is so high that it lowers the potential rate of lipogenesis by feedback inhibition. Moreover, substances that can transport reductive hydrogens into the mitochondria and thus remove this excess should stimulate the incorporation of lactate into lipid. Such stimulation has been observed in rat adipose tissue by tetra-methyl-p-phenylenediamine (TMPD; Halperin and Robinson, 1970) and by phenazine methosulfate (PMS; Katz and Wals, 1970). The effect of these two compounds on glucose and lactate incorporation was studied (Table VII). Both compounds were without significant effect on glucose, and whereas TMPD caused some stimulation of lactate incorporation, PMS caused dramatic inhibition. Thus reduction of cytosolic-$NADH_2$ in skin cells inhibits rather than stimulates lipogenesis from lactate. In this, skin differs from other

¶ Hodgins, L. T. (1972). Aspects of cutaneous lipogenesis: With special reference to the synthesis of odd-numbered and branched-chain fatty acids. Thesis for the degree of Ph.D., New York University.

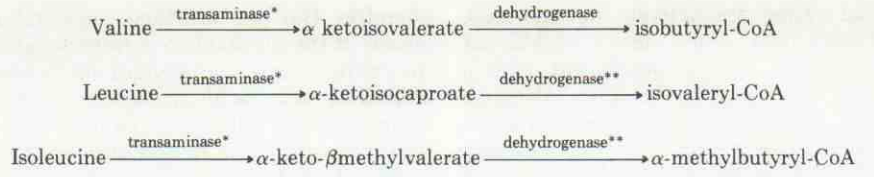

FIG. 3: Pathways of incorporation of branched amino acids into branched-chain fatty acids (Lipkin et al., 1965).
* These transaminases are identical and require α-ketoglutarate as an amino acceptor.
** These dehydrogenases are identical and differ from α-ketoisovalerate dehydrogenase.

TABLE VI

Generation of lactate during skin slice incubation

	Percentage of total incorporated activity
Distribution of incorporated activity after incubation with D-glucose-^{14}C	
Carbon dioxide	5.69
Lipids	2.02
Lactate	88.32
Tissue (as protein, etc.)	3.95
	mMolar
Lactate concentrations after 4 hr	
Tissue	40.76
Medium	1.03

The above results represent the mean of duplicate incubations performed on ear slices from two different animals.

TABLE VII

Effects of TMPD and PMS on glucose and lactate incorporation

Precursor	Effector	Concentration (mM)	Incorporation rate as a percentage of control incubation	
			Fatty acids	Non-saponifiables
Glucose-U-^{14}C	TMPD	0.05	91.2*	98.7
	PMS	0.01	84.4	72.6
D,L-Lactate-2-^{14}C	TMPD	0.02	148.7	111.8
	TMPD	0.05	144.3	106.0
	PMS	0.01	17.2	16.2
	PMS	0.005	60.7	42.6

* As in Table II. For abbreviations see text.

tissues and so it may be able to utilize lactate more efficiently as a precursor of lipids.

The entire subject of lactate utilization merits active investigation. Inconsistencies in the data need to be explained. Moreover, the validity of the skin slice technique as a method of evaluating cutaneous lipogenesis may be open to criticism, and the use of the sebaceous complexes of rodents may be contraindicated. For example, some cutaneous complexes such as the gerbil ventral gland have a thin overlying layer of epidermis; preputial glands, on the other hand, are located remotely from the skin. Therefore, the two types of sebaceous structures may show different responses both in vivo and in vitro, and probably neither responds in the same way as the true sebaceous glands of these animals. If it is essential that epidermis and sebaceous glands coexist, this could explain the variable results obtained with these types of tissues (Wheatley et al., 1970). Until the situation is clarified, data obtained from the study of isolated sebaceous glands should be interpreted with caution (Summerly and Woodbury, 1971).

The Role of Glucose

In all of the precursor studies, the addition of glucose to the media stimulates lipogenesis (Table VIII), the degree of stimulation varying from about twofold with pyruvate and lactate to about 16-fold with isoleucine. Since the rate of lipogenesis in skin slices increases with the increasing concentration of substrate (Wheatley et al., 1971), this effect with lactate, and perhaps to some extent with pyruvate, could be due to the additional substrate generated by glycolysis. Nevertheless, glucose may play an essential role in cutaneous lipogenesis, and attempts were made to establish this role. In addition to the generation of pyruvate, glucose can generate $NADPH_2$ (by the HMP-shunt), $NADH_2$, and α-GP. The addition of α-GP, either with or without preincubation, was completely without effect on acetate incorporation (this should be distinguished from the studies of Ziboh and Hsia [1969] who used rat abdominal skin). To some extent, pyruvate and, more markedly, lactate stimulated acetate incorporation, but the effect was appreciably less than that of glucose. To determine whether the effect of glucose on pyruvate was due to the generation of $NADH_2$, the addition of glyceraldehyde phosphate (GAP) was studied. GAP, the precurosr for $NADH_2$ generation in glycolysis, could generate pyruvate equally as well. However, it was without effect on either acetate or pyruvate incorporation, either because the stimulating effect of glucose is due to the activity of the HMP-shunt or because skin is impermeable to α-GP and GAP.

All these data suggest that skin preferentially utilizes glucose for lipogenesis. However, in vivo, the skin must rely on the precursors available in the tissue fluids in proximity to the cells undergoing lipogenesis. In view of the high metabolic ac-

tivity of these cells, there is bound to be competition for the available glucose between lipogenesis and other metabolic processes. The studies of the behavior of acetate, pyruvate, and lactate (see above), almost in contradiction to the observations just described, show that active lipogenesis proceeds in skin even in the absence of glucose. This suggests that mechanisms in cutaneous lipogenesis are so adaptable that they can accept any available precursor. Perhaps this natural adaptability of cutaneous lipogenesis precludes the homeostatic responses observed in other tissues. In other words, the skin can adapt its lipogenic pathways to take the best advantage of available precursors and does not stimulate or suppress lipogenesis as precursor availability varies. Only when precursor availability reaches extreme porportions does the skin, like any other starved organism, respond (Pochi et al., 1970).

TABLE VIII

Effects of glucose and glycolysis products on incorporation rates

Precursor	Effector	Incorporation rate as a percentage of control incubation	
		Fatty acids	Nonsaponifiables
Acetate-1,2-^{14}C	Glucose	324.8*	215.3
	α-GP	101.6	132.7
	α-GP (preincubated)	103.8	109.4
	GAP	91.9	75.7
	Pyruvate	126.0	123.5
	Lactate	180.8	130.2
Pyruvate-2-^{14}C	Glucose	178.8	168.3
	GAP	87.6	97.8
D,L-Lactate-2-^{14}C	Glucose	266.6	163.4
Isoleucine-U-^{14}C	Glucose	1640.0	572.4
Leucine-U-^{14}C	Glucose	834.5	202.1

As in Table II. For abbreviations see text.

PATHWAY ANALYSIS

To elucidate some specific aspects of pathway flow during cutaneous lipogenesis, studies of the incorporation of specifically labelled aspartate and glutamate were undertaken. In planning these experiments, we duplicated those of Leveille and Hanson (1966) in their study of rat adipose tissue so that data would be directly comparable. Aspartate can be incorporated into lipid only after the oxaloacetate, formed after transamination, is decarboxylated; thus only C-2 and C-3 are incorporated. The metabolism of specific carbon atoms of glutamate is more complicated and, for details, the reader is referred to the excellent illustration presented by Leveille and Hanson (Fig. 3, in the reference just cited). A brief summary is as follows:

C-1 Carbon dioxide only
C-2 Recycled as oxaloacetate formed by reverse Krebs cycle
C-3 From oxaloacetate formed in both the forward and reverse Krebs cycles
C-4 From both forward and reverse Krebs cycles
C-5 From reverse Krebs cycle only

The results, obtained with skin from guinea pig ear, are shown in Table IX. The incorporation of aspartate and glutamate into lipid is relatively low in skin, being less than 5 percent of that incorporated into carbon dioxide. In adipose tissue, this

TABLE IX

Incorporation of aspartate, glutamate, and citrate

Precursor	Incorporation rate into:		
	Fatty acids (nmoles/gm/hr)	Nonsaponifiables (nmoles/gm/hr)	Carbon dioxide (nmoles/gm/hr)
L-Aspartate-U-^{14}C	9.0 ± 0.3	3.4 ± 0.2	276.6 ± 23.9
L-Glutamate-U-^{14}C	5.2 ± 0.1	2.0 ± 0.1	206.9 ± 2.1

	Incorporation rate into fatty acids			
	Animal A (nmoles/gm/hr)	Animal B (nmoles/gm/hr)	Animal C (nmoles/gm/hr)	Animal D (nmoles/gm/hr)
Acetate-1,2-^{14}C	70.24 ± 5.65	106.71 ± 4.93	114.36 ± 7.68	45.07 ± 5.61
D,L-Asparate-3-^{14}C	3.06 ± 0.28	2.52 ± 0.33	—	—
D,L-Asparate-4-^{14}C	0.23 ± 0.01	0.01 ± 0.03	—	—
Citrate-1,5-^{14}C	2.78 ± 0.28	2.82 ± 0.10	—	—
D,L-Glutamate-1-^{14}C	0.17 ± 0.03	0.16 ± 0.01	—	—
D,L-Glutamate-2-^{14}C	0.40 ± 0.01	0.40 ± 0.01	0.70 ± 0.07	0.45 ± 0.08
D,L-Glutamate-5-^{14}C	1.38 ± 0.06	2.30 ± 0.23	3.27 ± 0.24	1.05 ± 0.12
D,L-Glutamate-3,4-^{14}C	—	—	12.76 ± 0.89	4.96 ± 0.48

The above results represent the mean and standard error of triplicate incubations. Precursors of higher specific activity were used in these experiments and rates >0.2 are significant.

proportion is much higher even though incorporation into carbon dioxide by both tissues is comparable. Obviously both compounds are actively metabolized by skin mitochondria although they are poor precursors for lipogenesis in skin. The incorporation of glutamate-5-^{14}C is measurable; hence the activity of the reverse Krebs cycle (D'Adamo and Haft, 1965) is significant and accounts for some 23 percent of the total glutamate flux. As indicated by the incorporation of glutamate-2-^{14}C, an estimated 28 percent of the oxaloacetate liberated from citrate by the citrate cleavage enzyme is recycled, an indication of the general level of recycling oxaloacetate. Except for the relatively lower level of activity, these data support the contention that the pathways of aspartate and glutamate metabolism are similar for skin and adipose tissue.

The major pathways of carbon flow during cutaneous lipogenesis can now be summarized (Fig. 4). This flow pattern is consistent with the data presented, and some indications of relative flow rates along specific pathways have already been suggested; they are summarized in Table X. Some of the data in this table are no better than an educated guess and should be treated as such; they are presented merely as guides for future work.

QUESTIONS, ENIGMAS, AND CONUNDRUMS

A question concerning the adaptive responses in skin was posed at the beginning of this paper and some suggestions were made, but the question remains unanswered. A related enigma concerning the effect of insulin on cutaneous lipogenesis has also engaged our energies. The results indicate that insulin is without effect on lipogenesis in guinea pig skin (Wheatley et al., 1971), yet Hsia et al. (1966) present convincing evidence that it is effective in human skin. Does this disparity represent species differences or is there an alternative explanation?

Here are a few other questions. How are the mono- and diester waxes formed? We can propose mechanisms that involve the reduction of fatty acids to alcohols (Sand et al., 1969), but we have no evidence that these are active in skin. Why does the epidermis generate so much lactate? Are the

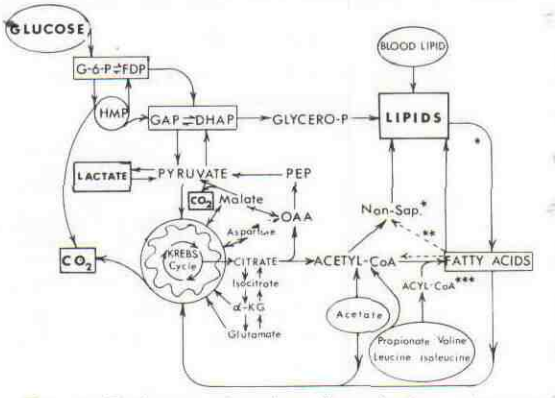

FIG. 4: Pathways of carbon flow during cutaneous lipogenesis. The points of entry of acetate and propionate are indicated although it is unlikely that these are physiologic precursors. Propionyl-CoA is most likely derived from isoleucine. Metabolic pools are enclosed in boxes. Each box does not necessarily represent a single pool. The heterogeneous nature of skin makes multiple pools inevitable. *Abbreviations:* see Fig. 1 and text.

* Major components are sterols and related compunds, wax alcohols and alkane-1,2-diols.

** Comparative analytical data (Nicolaides, 1963) suggest that the wax alcohols and alkane-diols are derived from fatty acids, but direct confirmation is lacking.

*** These comprise propionyl-CoA, isobutyryl-CoA, isovaleryl-CoA, methylbutyryl-CoA and possibly methylmalonyl-CoA. Malonyl-CoA is not included.

TABLE X

Relative rates in various pathways of carbon flow

Glucose flux		
To lactate		88% of the total glucose flow
To lipid:		
as glyceride	0.5%	2% of the total glucose flow
as fatty acids and nonsaponifiables	1.5%	
To carbon dioxide		6% of the total glucose flow
To tissue, as protein, glycogen, etc		4% of the total glucose flow
HMP-shunt		3% of the total glucose uptake
Citrate cleavage pathway		
Cytosolic acetyl-CoA formed		90% of the citrate translocated
Cytosolic oxaloacetate recycled		30% of the citrate cleaved
Malate cycle		1% of the total pyruvate formation
Branched-chain amino acid utilization		
As terminal groups		20% of the total incorporated into lipid
As acetyl-CoA		80% of the total incorporated into lipid
Percentage of total fatty acid formation		5% of the total incorporated into lipid
Acetyl-CoA conversion		
To fatty acids		80% of the total lipid incorporation
To nonsaponifiables		20% of the total lipid incorporation

various pathways involved in synthesizing the various components of sebum synchronized so that the composition is maintained constant or are they random? What is the primer specificity of the fatty acid synthetase of skin? Is it primed only by acetyl-CoA, can it be primed by the branched and odd acyl-CoA compounds, or is butyryl-CoA the primer of choice? In the latter connection, butyrate is incorporated more rapidly than acetate (Table I). Does this mean that butyryl-CoA is the physiologic primer (Lin and Kumar, 1971) and not acetyl-CoA? Butyryl-CoA can be generated from 2 acetyl-CoA's via acetoacetyl-CoA and crotonyl-CoA by the pathway proposed by Lin and Kumar (1972).

Among the enigmas, we have the cutaneous metabolism of propionate, carbon dioxide fixation, and the role of the HMP-shunt. It is difficult to suggest feasible mechanisms for the first two, and the HMP-shunt enigma has resisted clarification. Concerning the latter, it is particularly relevant to consider the action of testosterone on the sebaceous gland. This hormone stimulates sebum production after it first stimulates HMP-shunt activity (Sansone et al., 1971); thus, at least by implication, it stimulates lipogenesis by this mechanism. But does testosterone really stimulate lipogenesis in the sebaceous gland? Testosterone is basically a growth hormone; and pentoses, produced by the HMP-shunt, are essential to form the nucleotides for cell replication in a growing system. The sebaceous gland is holocrine and must replicate to produce sebum. After replication, must lipogenesis in the sebaceous cell proceed at a predetermined rate until the cell ruptures at maturity, or does the rate vary to allow the cells to mature more rapidly or more slowly? Is the regulation of lipogenesis related to sebum secretion or is the latter controlled by the rate of cell replication alone? What is the effect of stimulating sebaceous cell replication in the presence of an inhibitor of lipogenesis? Is the sebocyte transformed to a keratinocyte under these conditions?

REFERENCES

Abraham, S., and Chaikoff, I. L. (1959). Glycolytic pathways and lipogenesis in mammary glands of lactating and nonlactating rats. J. Biol. Chem., *234:* 2246–2253.

Barker, S. B., and Summerson, W. H. (1941). The colorimetric determination of lactic acid of biological materials. J. Biol. Chem., *138:* 535–554.

Barron, E. S. G., Meyer, J., and Miller, Z. B. (1948). The metabolism of skin. Effect of vesicant agents. J. Invest. Dermatol., *11:* 97–118.

Bowden, J. A., and Connelly, J. T. (1968). Branched-chain α-keto acid metabolism. II. Evidence for the common identity of α-keto-isocaproic acid and α-keto-β-methylvaleric acid dehydrogenases. J. Biol. Chem., *243:* 3526–3531.

Bush, E. T. (1963). General applicability of the channels ratio method for measuring liquid scintillation efficiencies. Anal. Chem., *35:* 1024–1029.

Chappell, J. B. (1968). Systems used for the transport of substrates into mitochondria. Br. Med. Bull., *24:* 150–157.

Cruickshank, C. N. D., and Trotter, M. D. (1956). The oxygen uptake, glucose utilization and lactic acid production of guinea pig skin in relation to oxygen tension. Biochem. J., *62:* 57–61.

Cruickshank, C. N. D., Trotter, M. D., and Cooper, J. E. (1962). A study of available substrates for the endogenous respiration of skin "in vitro." J. Invest. Dermatol., *39:* 175–178.

D'Adamo, A. F., and Haft, D. E. (1965). An alternative pathway of α-ketoglutarate catabolism in the isolated, perfused rat liver. J. Biol. Chem., *240:* 613–616.

Decker, R. H. (1971). Nature and regulation of energy metabolism in epidermis. J. Invest. Dermatol., *57:* 351–363.

Donaldson, W. E., Wit-Peeters, E. M., and Scholte, H. R. (1970). Fatty acid synthesis in rat liver: Relative contributions of the mitochondrial, microsomal and non-particulate systems. Biochim. Biophys. Acta, *202:* 35–42.

Feller, D. D. (1954). Metabolism of adipose tissue. I. Incorporation of acetate carbon into lipides by slices of adipose tissue. J. Biol. Chem., *206:* 171–180.

Firscheim, H. E., and Bell, J. (1961). Metabolism of connective tissue lactic and citric acid formation by skin. J. Biol. Chem. *236:* 22–25.

Flatt, J. P. (1970). Conversion of carbohydrate to fat in adipose tissue: An energy yielding and, therefore self-limiting process. J. Lipid Res., *11:* 131–143.

Flatt, J. P., and Ball, E. G. (1964). Studies on the metabolism of adipose tissue. XV. An evaluation of the major pathways of glucose catabolism as influenced by insulin and epinephrine. J. Biol. Chem., *239:* 675–685.

Foster, D. W., and Bloom, B. (1963). The synthesis of fatty acids by rat liver slices in tritiated water. J. Biol. Chem., *238:* 888–892.

Freinkel, R. K. (1960). Metabolism of glucose-^{14}C by human skin *in vitro*. J. Invest. Dermatol., *34:* 37–42.

Garland, P. B. (1968). Control of citrate synthesis in mitochondria. In: *The Metabolic Role of Citrate* (ed. by Goodwin, T. W.) Academic Press Inc., London, pp. 41–60.

Grigor, M. R., Dunckley, G. G., and Purves, H. D. (1970). The synthesis of the branched-chain fatty acids of rat skin surface lipid. Biochim. Biophys. Acta, *218:* 389–399.

Halprin, K. M., and Ohkawara, A., (1966). Lactate production and lactate dehydrogenase in human epidermis. J. Invest. Dermatol., *47:* 222–226.

Halperin, M. L., and Robinson, B. H. (1970). The role of the cytoplasmic redox potential in the control of fatty acid synthesis from glucose, pyruvate and lactate in white adipose tissue. Biochem. J., *116:* 235–240.

Hanson, R. W., Patel, M. S., Jomain-Baum, M., and Ballard, F. J. (1971). Role of mitochondria in metabolism of pyruvate and lactate by rat adipose tissue. Metabolism *20:* 27–42.

Horning, M. G., Martin, D. B., Karmen, A., and Vagelos, P. R. (1961). Fatty acid synthesis in adipose tissue. II. Enzymatic synthesis of branched-chain and odd-numbered fatty acids. J. Biol., Chem., *236:* 669–672.

Howard, C. F., and Lowenstein, J. M. (1965). The effect of glycerol 3-phosphate on fatty acid synthesis. J. Biol. Chem., *240:* 4170–4175.

Hsia, S. L., Dreize, M. A., and Marquez, M. C. (1966). Lipid metabolism in human skin. II. A study of lipogenesis in skin of diabetic patients. J. Invest. Dermatol., *47:* 443–448.

James, A. T., Peeters, G., and Lauryssens, M. (1956). Metabolism of propionic acid. Biochem. J., *64:* 726–730.

James, A. T., and Wheatley, V. R. (1956). Studies of sebum. 6. The determination of the component fatty acids of human forearm sebum by gas-liquid chromatography. Biochem. J., *63:* 269–273.

Jungas, R. L., (1968). Fatty acid synthesis in adipose

tissue incubated in triated water. Biochemistry, *7:* 3708–3717.

Kandutsch, A. A. (1964). Sterol metabolism in skin and epidermis. In: *The Epidermis* (ed. by Montagna, W., and Lobitz, W. C., Jr.). Academic Press Inc., New York, pp. 403–510.

Katz, J., and Kornblatt, J. (1962). Propionate metabolism by slices of mammary gland of lactating rat. J. Biol. Chem., *237:* 2466–2473.

Katz, J., and Wals, P. A. (1970). Effect of phenazine methosulfate on lipogenesis. J. Biol. Chem., *245:* 2546–2548.

Kneer, P., and Ball, E. G. (1968). Studies on the metabolism of adipose tissue. XXI. An evaluation of the major pathways of pyruvate metabolism. J. Biol. Chem., *243:* 2863–2870.

Landau, B. R., and Katz, J. (1965). Pathways of glucose metabolism. In: *Handbook of Physiology, Section 5, Adipose Tissue* (ed. by Renold, A. E., and Cahill, G. F.). American Physiological Society, Washington, D.C., pp. 253–271.

Leveille, G. A., and Hanson, R. W. (1966). Adaptive changes in enzyme activity and metabolic pathways in adipose tissue from meal-fed rats, J. Lipid Res., *7:* 46–55.

Lin, C. Y., and Kumar, S., (1971). Primer specificity of mammary gland fatty acid synthetase and the role of the soluble β-oxidative enzymes. J. Biol. Chem., *246:* 3284–3290.

Lin, C. Y., and Kumar, S. (1971). Primer specificity of synthesis of fatty acids in mammalian tissues. J. Biol. Chem., *247:* 604–606.

Lipkin, G., Wheatley, V. R., and March, C. (1965). Studies of the lipids of dog skin. III. The *in vivo* incorporation of acetate, pyruvate and certain amino acids into the lipids of isolated perfused dog skin. J. Invest. Dermatol., *45:* 356–361.

Lowenstein, J. M. (1968). Citrate and the conversion of carbohydrate into fat. In: *The Metabolic Role of Citrate* (ed. by Goodwin, T. W.). Academic Press Inc., London, pp. 61–86.

Masoro, E. J. (1968). *Physiological Chemistry of Lipids in Mammals.* W. B. Saunders Co., Philadelphia, pp. 58–73.

Mier, P. D. (1969). The carbohydrate metabolism of skin. Br. J. Dermatol., *18*(Suppl 2)*:* 14–17.

Nicolaides, N. (1963). Human skin surface lipids—origin, composition and possible function. In: *Advances in Biology of Skin. Vol. 4. The Sebaceous Glands* (ed. by Montagna, W., Ellis, R. A., and Silver, A. F.). Pergamon Press, New York, pp. 135–147.

Nicolaides, N., Fu, H. C., and Ansari, M. N. A. (1970). Diester waxes in surface lipids of animal skin. Lipids, *2:* 299–307.

Patel, M. S., Jomain-Baum, M., Ballard, F. J., and Hanson, R. W. (1971). Pathway of carbon flow during fatty acid synthesis from lactate and pyruvate in rat adipose tissue. J. Lipid Res., *12:* 179–191.

Pochi, P. E., Downing, D. T., and Strauss, J. S. (1970). Sebaceous gland response in man to prolonged total caloric deprivation. J. Invest. Dermatol., *55:* 303–309.

Robinson, B. H., and Halperin, M. L. (1970). Transport of reduced nicotinamide-adenine dinucleotide into mitochondria of rat adipose tissue. Biochem. J., *116:* 229–233.

Sand, D. M., Hehl, J. L., and Schlenk, H. (1969). Biosynthesis of wax esters in fish. Reduction of fatty acids and oxidation of alcohols. Biochemistry, *8:* 4851–4854.

Sansone, G., Davidson, W., Cummings, B., and Reisner, R. M. (1971). Sebaceous gland lipogenesis induced by testoserone: Early metabolic events. J. Invest. Dermatol., *57:* 144–148.

Shrago, E., Glennon, J. A., and Gordon, E. S. (1971). Comparative aspects of lipogenesis in mammalian tissues. Metabolism, *20:* 54–62.

Sullivan, A. C., Hamilton, J. G., Miller, O. N., and Wheatley, V. R. (1972). Inhibition of lipogenesis in rat liver by (−)-hydroxycitrate. Arch. Biochem., *150:* 183–190.

Summerly, R., and Woodbury, S. (1971). The *in vitro* incorporation of ^{14}C-acetate into the isolated sebaceous gland and appendage free epidermis of human skin. Br. J. Dermatol., *85:*424–431.

Taylor, R. T., and Jenkins, W. T. (1966). Leucine aminotransferase. II. Purification and characterization. J. Biol. Chem., *241:* 4396–4405.

Wheatley, V. R., Chow, D. C., and Keenan, F. D. (1961). Studies of the lipids of dog skin. II. Observations on the lipid metabolism of perfused surviving dog skin. J. Invest. Dermatol., *36:* 237–239.

Wheatley, V. R., and Hodgins, L. T. (1968). Utilization of circulating lipids for cutaneous lipid formation. J. Invest. Dermatol., *50:* 271.

Wheatley, V. R., Hodgins, L. T., and Coon, W. M. (1970). Cutaneous lipogenesis. I. Evaluation of model systems and the utilization of acetate, citrate and glucose as compared with other tissues. J. Invest. Dermatol., *54:* 288–297.

Wheatley, V. R., Hodgins, L. T., Coon, W. M., Kumarasiri, M. Berenzeig, H., and Feinstein, J. M. (1971). Cutaneous lipogenesis: Precursors utilized by guinea pig skin for lipid synthesis. J. Lipid Res., *12:* 347–360.

Wheatley, V. R., and James, A. T. (1957). Studies of sebum. 7. The composition of the sebum of common rodents. Biochem. J., *65:* 36–42.

Wheatley, V. R., Lipkin, G., and Woo, T. H. (1967). Lipogenesis from amino acids in perfused isolated dog skin. J. Lipid Res., *8:* 84–89.

Yardley, H. J., and Godfrey, G. (1963). Direct evidence for the hexose monophosphate pathway of glucose metabolism in skin. Biochem. J., *86:* 101–103.

Ziboh, V. A., and Hsia, S. L. (1969). Lipogenesis in rat skin: A possible regulatory role of glycerol-3-phosphate. Arch. Biochem., *131:* 153–162.